

Claims

1. An adjuvant comprising an isolated conjugate of a CD28 and/or CD40 antibody and at least one antigen wherein said conjugate consists of an oligomeric
5 complex wherein the antibody valency of the complex does not exceed an average of about five antibody molecules per complex.
2. An adjuvant according to Claim 1 wherein said conjugate consists an average of one to four antibody molecules per complex.
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3. An adjuvant according to Claim 2 wherein said complex consists an average of one to three antibody molecules per complex.
4. An adjuvant according to Claim 2 or 3 wherein said complex consists of one
15 to two antibody molecules per complex.
5. A vaccine composition comprising a conjugate according to any of Claims 1-4.
- 20 6. A vaccine according to Claim 5 wherein said composition further comprises a carrier.
7. A vaccine according to Claim 5 or 6 wherein said composition further comprises a second adjuvant.
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8. An vaccine according to Claim 7 wherein said composition comprises a mixture of a CD40 adjuvant and a CD28 adjuvant according to any of Claims 1-4.
9. An adjuvant according to any of Claims 1-4 wherein said antigen is a T-cell
30 dependent antigen.

10. An adjuvant according to any of Claims 1-4 wherein said antigen is a T- cell independent antigen.
11. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from
5 a pathogenic bacterium.
12. An adjuvant according to Claim 11 wherein said antigen is derived from a bacterial species selected from the group consisting of: *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Enterococcus faecalis*; *Mycobacterium tuberculosis*;
10 *Streptococcus group B*; *Streptococcus pneumoniae*; *Helicobacter pylori*; *Neisseria gonorrhea*; *Streptococcus group A*; *Borrelia burgdorferi*; *Coccidioides immitis*; *Histoplasma sapsulatum*; *Neisseria meningitidis* ; *Shigella flexneri*; *Escherichia coli*; *Haemophilus influenzae*.
13. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from
15 a viral pathogen.
14. An adjuvant according to Claim 13 wherein said antigen is derived from a viral pathogen selected from the group consisting of: Human Immunodeficiency
20 Virus (HIV1 & 2); Human T Cell Leukaemia Virus (HTLV 1 & 2); Ebola virus; human papilloma virus (e.g. HPV-2, HPV-5, HPV-8 HPV-16, HPV-18, HPV-31, HPV-33, HPV-52, HPV-54 and HPV-56); papovavirus; rhinovirus; poliovirus; herpesvirus; adenovirus; Epstein Barr virus; influenza virus, hepatitis B and C viruses.
15. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from
25 a parasitic pathogen.
16. An adjuvant according to Claim 15 wherein said antigen is derived a
30 parasitic pathogen selected from the group consisting of: *Trypanosoma spp*, *Schistosoma spp* or *Plasmodium spp*.

17. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from a fungal pathogen.
- 5 18. An adjuvant according to Claim 17 wherein said antigen is derived from a fungal pathogen which is of the genus *Candida spp.*
19. An adjuvant according to Claim 9 or 10 wherein said antigen is a tumour specific antigen or a tumour associated antigen.
- 10 20. An adjuvant according to Claim 19 wherein said antigen is a ganglioside antigen.
21. An adjuvant according to Claim 19 or 20 wherein said antigen is MUC-1.
- 15 22. An adjuvant according to Claim 9 or 10 wherein said antigen is a hormone or hormone receptor.
23. An adjuvant according to Claim 22 wherein said antigen is the N-methyl-D aspartate receptor, or part thereof.
- 20 24. An adjuvant according to Claim 9 or 10 wherein said antigen is a prion protein.
- 25 25. An adjuvant according to Claim 24 wherein said antigen is an amyloid protein.
26. An adjuvant according to Claim 25 wherein wherein said antigen is amyloid β or part thereof.
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27. An adjuvant according to Claim 9 or 10 wherein said antigen is a sperm antigen.
28. An adjuvant according to Claim 9 or 10 wherein said antigen is an addictive
5 drug.
29. An adjuvant according to Claim 28 wherein said drug is selected from the group consisting of: cocaine; nicotine; or heroin.
- 10 30. A method to immunise an animal to an antigen, comprising administering an effective amount of a conjugate according to any of Claims 1-29 sufficient to stimulate an immune response to said antigen.
31. A method according to Claim 30 wherein said animal is human.
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32. A method according to Claim 30 wherein said animal is selected from the group consisting of: mouse; rat; hamster; goat; cow, horse, pig, dog, cat or sheep.
33. A method according to any of Claims 30-32 wherein said route of
20 administration is intradermal, subcutaneous, intramuscular or intranasal.
34. A method according to Claim 33 wherein said route is intranasal.
35. An antibody obtainable by the method according to any of Claims 30-34.
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36. An antibody according to Claim 35 wherein said antibody is a monoclonal antibody or binding fragment thereof.
37. An antibody according to Claim 36 wherein said antibody is a humanised
30 antibody.

38. An antibody according to Claim 36 wherein said antibody is a chimeric antibody.

39. An antibody according to any of Claims 36-38 wherein said antibody is an opsonic antibody.

40. A method for preparing a hybridoma cell-line producing monoclonal antibodies according to Claim 36 comprising the steps of:

- i) immunising an immunocompetent mammal with a conjugate or, composition according 1-29;
- ii) fusing lymphocytes of the immunised immunocompetent mammal with myeloma cells to form hybridoma cells;
- iii) screening monoclonal antibodies produced by the hybridoma cells of step (ii) for binding activity to the antigen of the conjugate;
- iv) culturing the hybridoma cells to proliferate and/or to secrete said monoclonal antibody; and
- v) recovering the monoclonal antibody from the culture supernatant.

41. A hybridoma cell-line obtainable by the method according to Claim 40.

42. A method to crosslink an antibody, wherein said antibody is capable of binding a CD28 or CD40 receptor polypeptide, and at least one antigen characterised in that reaction conditions are provided which select for conjugates with low antibody valency.

43. A method to prepare an adjuvant according to any of Claims 1-4 or 9-29 comprising fractionation of a conjugation reaction mixture.

44. A method according to Claim 43 said fractionation comprises the following steps:

- i) providing a reaction mixture consisting of a heterogeneous crosslinked antibody: antigen conjugate complex;
- ii) separating the reaction mixture into fractions containing conjugates of defined size; and optionally
- 5 iii) isolating conjugates with a desired antibody valency.

45. A method according to Claim 44 wherein said fraction contains a conjugate complex with an antibody valency of about on average five antibody molecules per complex.

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46. A method according to Claim 44 wherein said fraction contains a complex with an antibody valency of between one to four antibodies per complex.

47. A method according to Claim 44 wherein said fraction contains a complex
15 with an antibody valency of between one and three antibodies per complex.

48. A method according to Claim 44 wherein said fraction contains a complex of two antibody molecules.

20 49. A method according to Claim 44 wherein said conjugate is a single antibody linked to at least one antigen.

50. A conjugate obtainable by the fractionation method according to any of Claims 43-49.

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